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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C12N 15/12, C07K 14/475, C12N 15/11, 15/62, C07K 16/22, C12Q 1/68, G01N 33/53</p>	A1	<p>(11) International Publication Number: WO 99/43811</p> <p>(43) International Publication Date: 2 September 1999 (02.09.99)</p>
<p>(21) International Application Number: PCT/US99/04142</p> <p>(22) International Filing Date: 25 February 1999 (25.02.99)</p> <p>(30) Priority Data: 60/075,922 25 February 1998 (25.02.98) US 60/079,567 27 March 1998 (27.03.98) US</p> <p>(71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): THORGEIRSSON, Snorri, S. [US/US]; 9305 Kingsley Avenue, Bethesda, MD 20814 (US). WOITACH, Joseph, T. [US/US]; 11613 Ashley Drive, North Bethesda, MD 20852 (US). ZHANG, Minghuang [CN/US]; 117 Bates Avenue, Gaithersburg, MD 20877 (US).</p> <p>(74) Agent: WOOD, William, J.; Merchant, Gould, Smith, Edell, Welter & Schmidt, P.A., 3100 Norwest Center, 90 South Seventh Street, Minneapolis, MN 55402-4131 (US).</p>	<p>(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: cDNA ENCODING A GENE BOG (B5T OVER-EXPRESSED GENE) AND ITS PROTEIN PRODUCT</p>		
<p>(57) Abstract</p> <p>Nucleic acids that encode novel polypeptides, designated in the present application as "BOG" (<u>B</u>5T <u>O</u>ver-expressed <u>G</u>ene) are provided. BOG binds to pRb and is over-expressed in a number transformed rat liver epithelial (RLE) cell lines resistant to the growth inhibitory effect of TGF-β1 as well as in primary liver tumors. Compositions including BOG chimeras, nucleic acids encoding BOG and antibodies to BOG are also provided. Methods of using BOG to modulate pRb-protein interactions and to alter cellular phenotype are further provided.</p>		

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FIG. 1

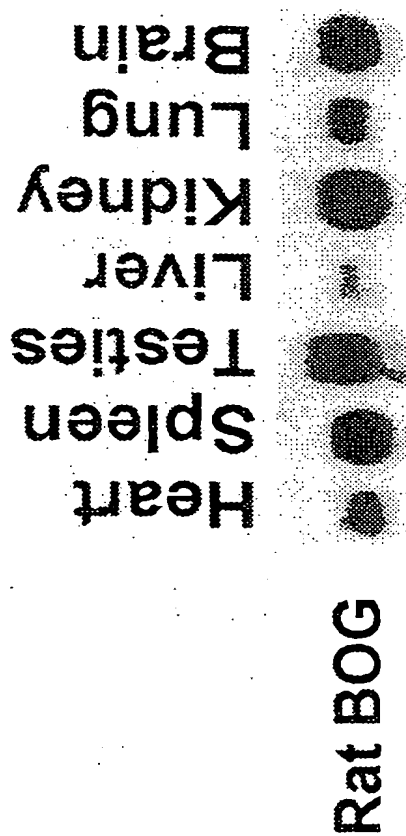


FIG. 2(A) A

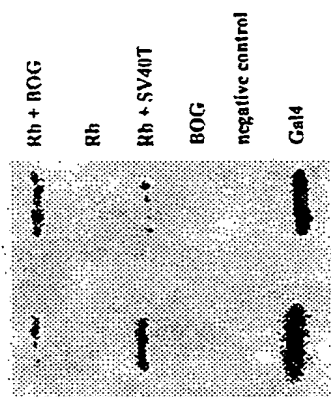


FIG. 2(B) B

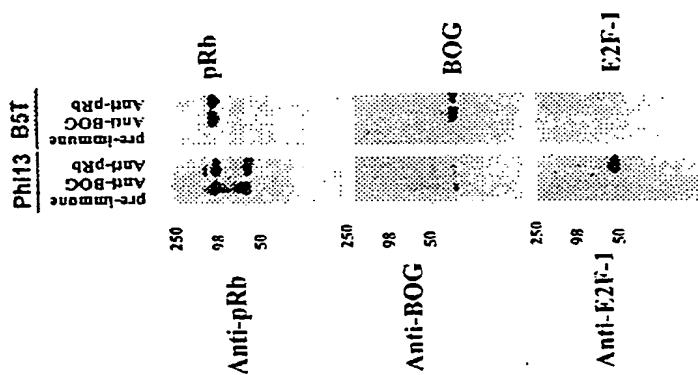


FIG. 2(C) C

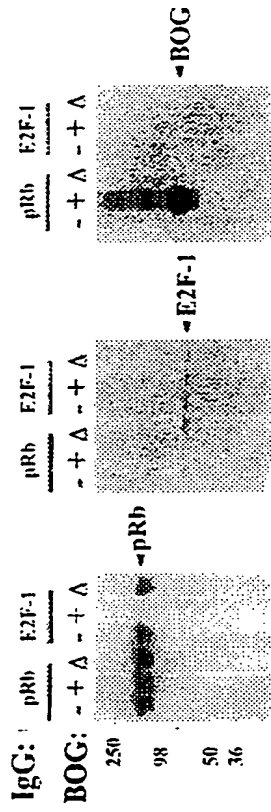


FIG. 2(D) D

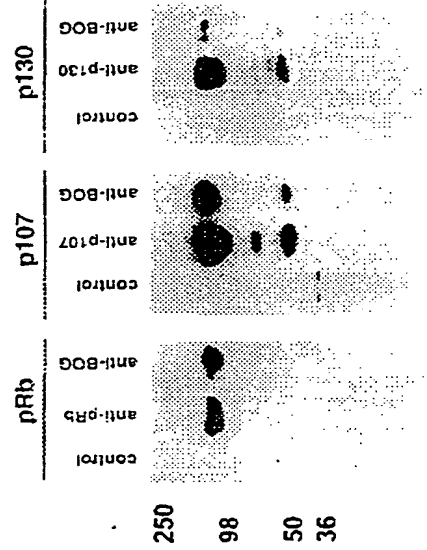


FIG. 3(A)

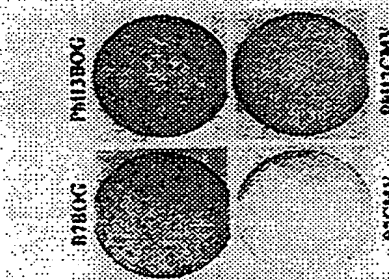


FIG. 3(C)

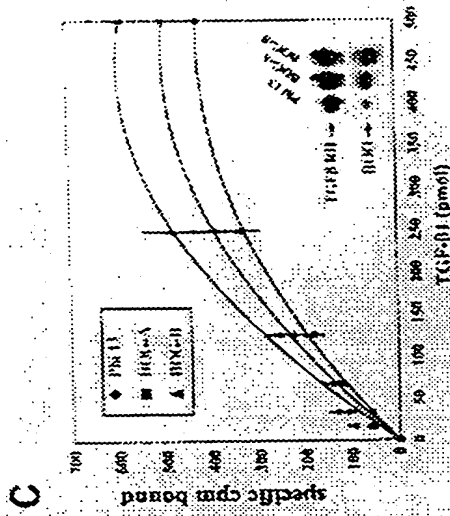


FIG. 3(B)

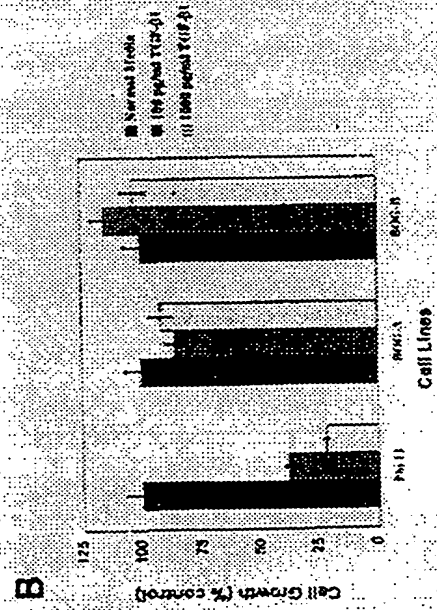
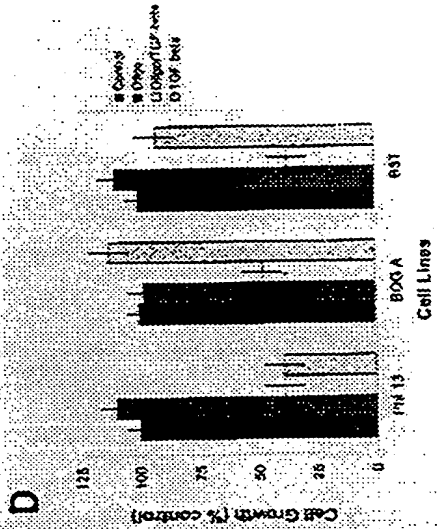


FIG. 3(D)



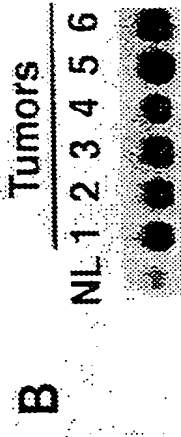
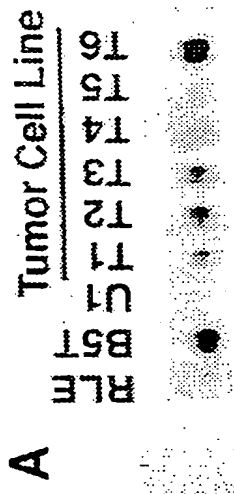


FIG. 5

BLAST SEARCH RESULTS

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2	Life Tech mouse embryo cDNA 10 5dpc clone 557151	AA111519 (mo51h08.r1)	1.6 e-146
3	Soares mouse 3NbMS cDNA clone 622827	AA184284 (m333b02.r1)	2.0 e-126
4	Stratagene mouse embryonic carcinomaRA cDNA clone 636882	AA105466 (mm92c06.r1)	1.0 e-120
5	WATMI Homosapiens cDNA clone 501115	N2288 (EST501115)	1.4 e-90

FIG. 6(B)



FIG. 6(A)

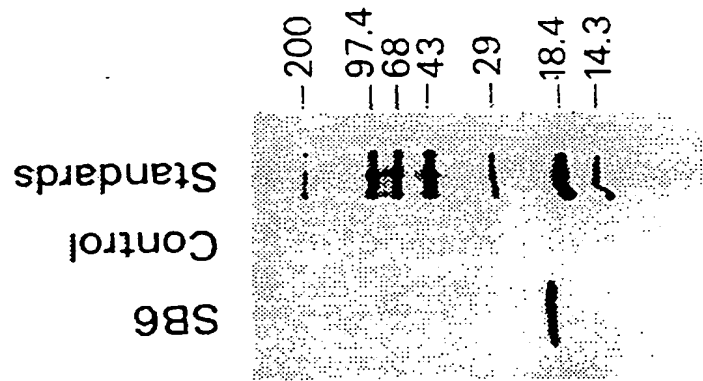


FIG. 7

Genomic Southern Blot

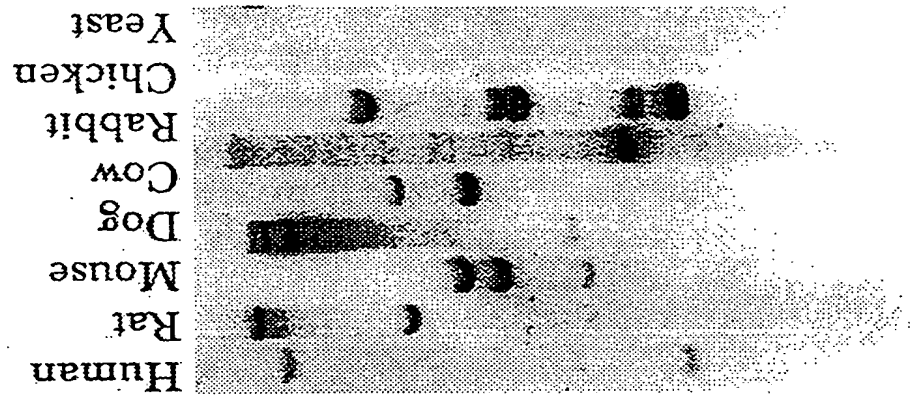


FIG. 8(A)

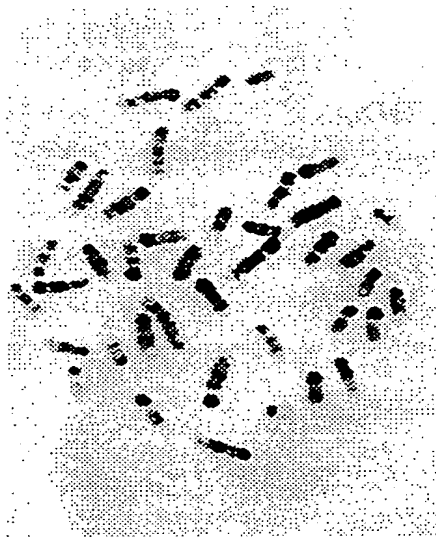


FIG. 8(B)

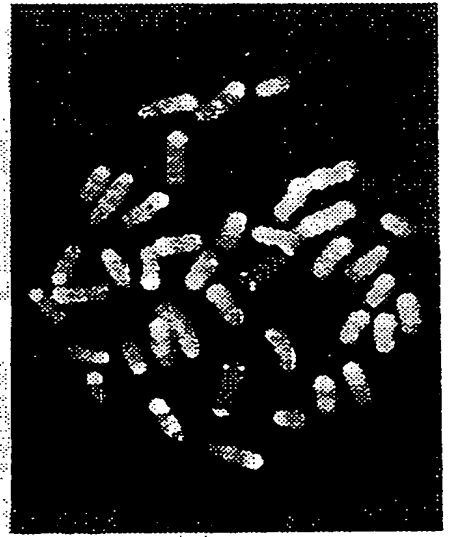


FIG. 8(C)

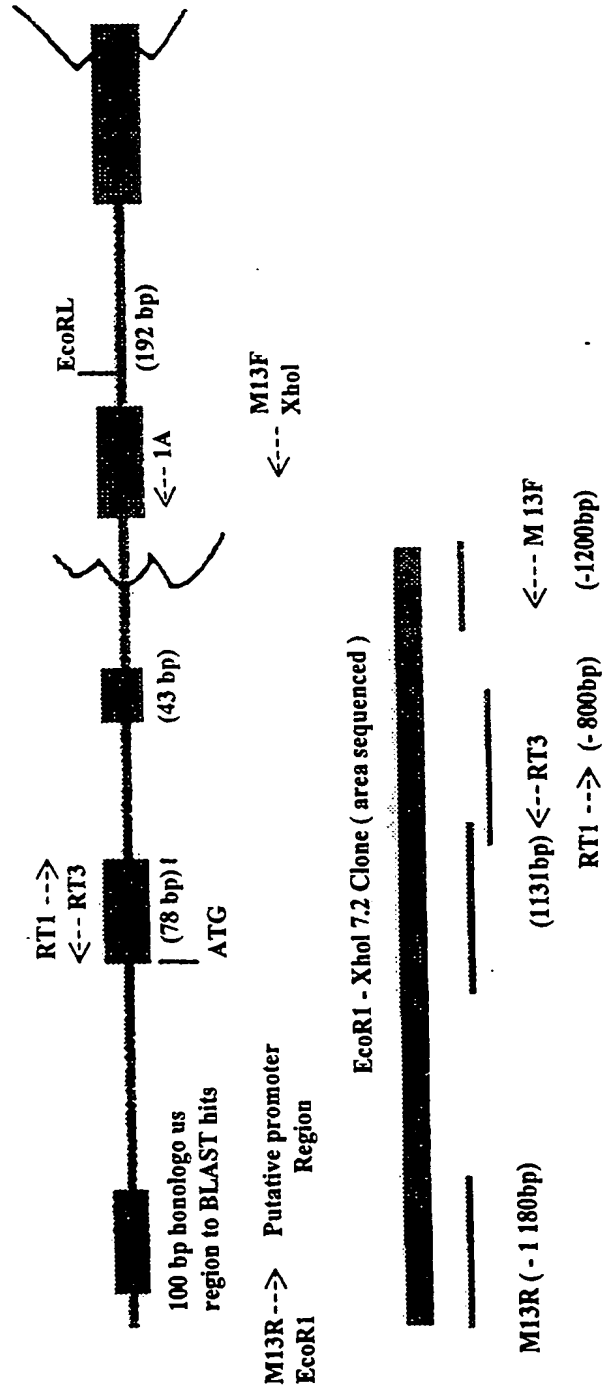


FIG. 9

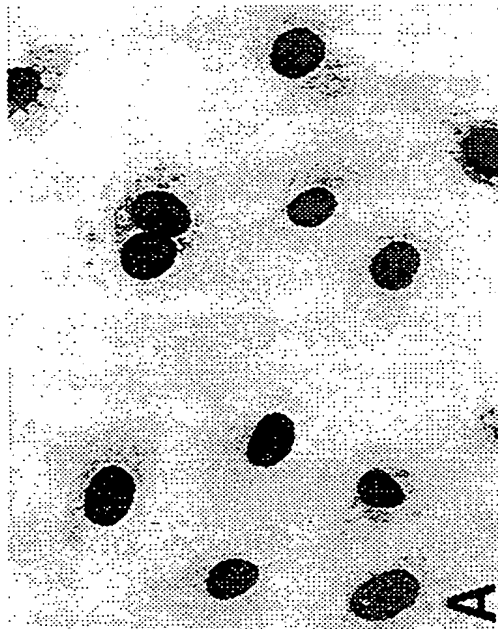


FIG. 9(A)

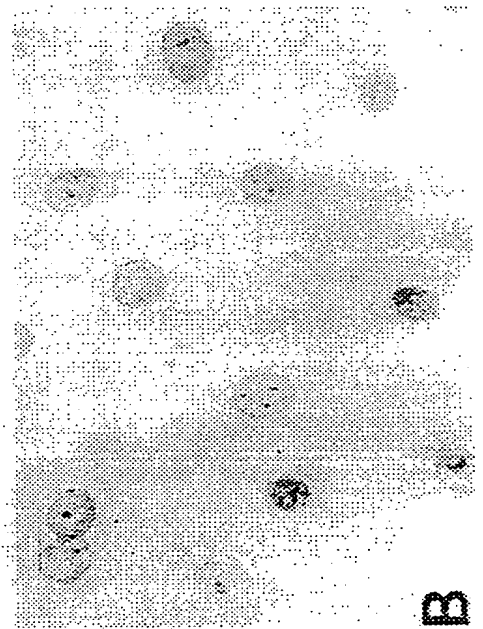


FIG. 9(B)

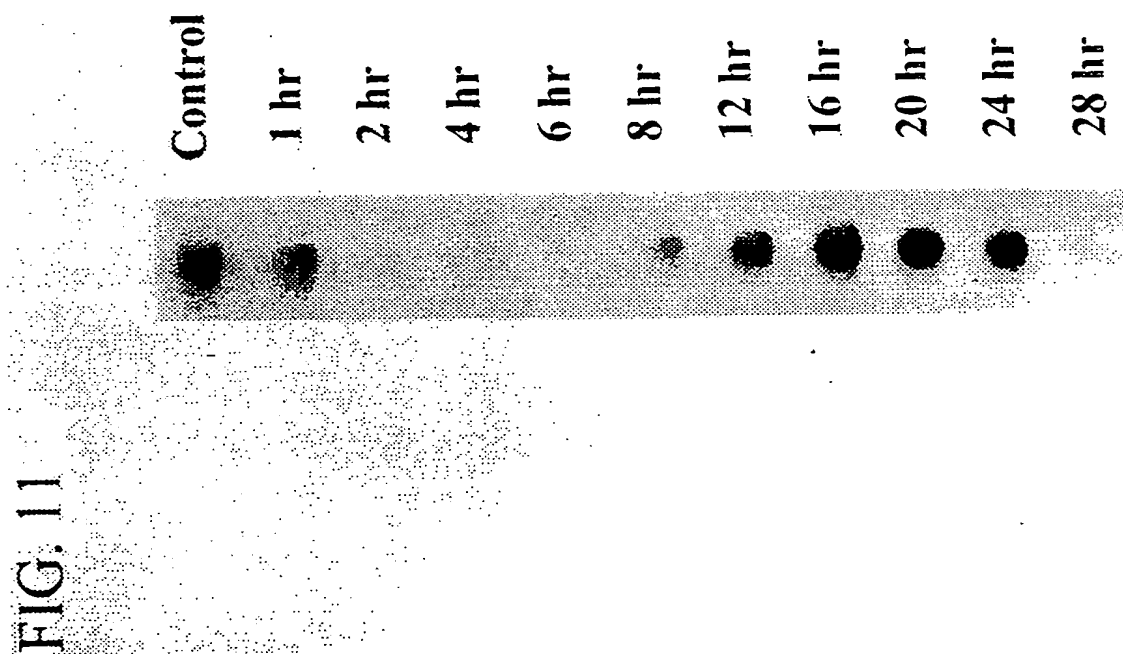


FIG. 12

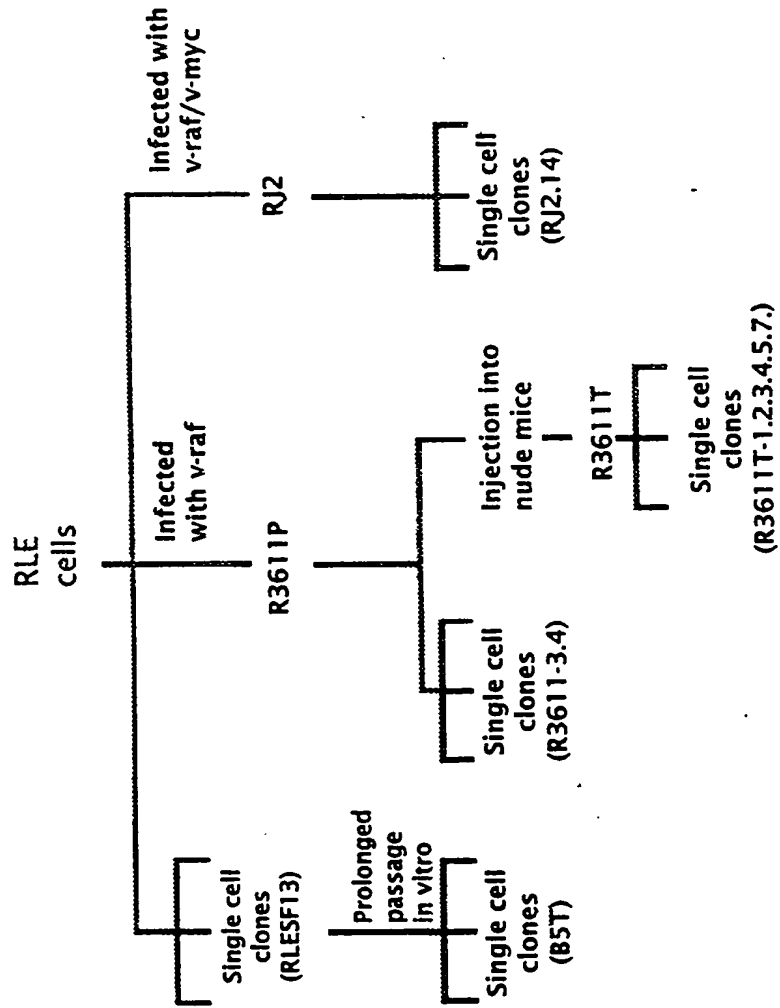


FIG. 13B

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<----- SRF (10.00 2.0000)
<----- TGGCA-binding protein (10.00 2.0000)
-----> HiNF-A (12.00 1.0000)
<----- CP2 (10.00 0.9091)
-----> IUF-1 (10.00 1.6667)
-----> HiNF-A (12.00 1.0000)
<----- AGP/EBP (10.00 1.1111)
<----- AP 3 (2) (13.00 1.6250)
<----- IqPE-1 (16.00 2.0000)
<----- LAP (10.00 1.1111)
<----- NF-IL6 (10.00 1.1111)
<----- NF-IL6beta (10.00 1.1111)

00201 GTGGAATGAAGGAAAAGAAGACACCCAACTGACTGAATATGGTGACA
<----- HSF (14.00 0.9333)
<----- HSF (14.00 0.9333)
<----- HSF (14.00 0.9333)
<----- HSF1 (long) (14.00 0.9333)
<----- HSF1 (short) (14.00 0.9333)

```

FIG. 13C

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```

<----- HSF1 (14.00 0.9333)
-----> c-Ets-2 (12.00 2.0000)
<----- c-Ets-1 54 (11.00 1.8333)
<----- c-Ets-1 68 (11.00 1.8333)
<----- c-Ets-2 58-64 (11.00 1.8333)
<----- PEA3 (11.00 1.8333)
<----- PEA3 (11.00 1.8333)
<----- PEA3 (11.00 1.8333)
-----> GR (12.00 2.0000)
<----- CACCC-binding factor (10.00 2.0000)
<----- gammaCAC1 (10.00 2.0000)
<----- gammaCAC2 (10.00 2.0000)
-----> AP-1 (16.00 2.0000)
-----> GCN4 (12.00 2.0000)
-----> delta factor (12.00 1.0909)
-----> YY1 (12.00 1.0909)
<----- myc-CF1 (10.00 1.6667)
-----> p300 (14.00 2.0000)
00251 CTCCCTTTTAATGCCAGCACTCAGGAGACAAAAGCAGGCAGATCTTTTG
-----> NF-1/L (10.00 2.0000)
-----> GCN4 (12.00 2.0000)
<----- Zeste (12.00 2.0000)
<----- Zeste (12.00 2.0000)
-----> ADRI (10.00 2.0000)
-----> LVc (10.00 2.0000)
-----> Zeste (10.00 2.0000)
-----> Zeste (10.00 2.0000)
00301 TGAGTTCTAGGCCAGTCTGGTTTACATAGACAGCTCCAGGCCAGTAAGGG
<----- GR (12.00 2.0000)
-----> NF-IL-2A (16.00 1.6000)
-----> Oct-1 (16.00 1.6000)
-----> Oct-2.1 (16.00 1.6000)
<----- VBP (20.00 1.6667)
-----> NF-E (10.00 2.0000)
<----- T-Ag (10.00 2.0000)
-----> E4BP4 (12.00 1.2000)
00351 GCTAGCTAATGAAACTGTCTTAACAATAACCAACGTTTCATTTGAAAA
-----> IUF-1 (10.00 1.6667)
<----- STE12 (16.00 2.0000)
<----- NF-E (10.00 2.0000)
<----- Ftz (18.00 1.6364)
-----> HOXA5 (10.00 1.0000)
-----> c-Ets-2 (12.00 2.0000)
<----- ECR (11.00 1.0000)
-----> TEID (12.00 2.0000)
-----> Hb (20.00 2.0000)
00401 AAAATAAACCTTCCTTAAGAAGTATTGGTACAATAAAAAAGATAAC
-----> AGP/EBP (10.00 1.1111)

```


FIG. 13A

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Sequence

RLHEXRT-3

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00001  CAGAGCCCTGAAAGGTTGTTGCATGAGCCCGTGAAAGTGGAGTTTCAGTG
          <----- NF-W1 (16.00 2.0000)
          <----- NF-W2 (16.00 2.0000)
          <---- Zeste (12.00 2.0000)
          <---- Zeste (12.00 2.0000)
          -----> BAF1 (10.00 0.8333)

00051  GTAGTGGATAGCATAGGACACTGGAGACACAGTTCATGTCCAGCATTTCAT
          <----- C/EBP (13.00 1.6250)
          <----- C/EBP (13.00 1.6250)
          <----- C/EBP (13.00 1.6250)
          <----- C/EBP (13.00 1.6250)
          ----> NF-E (10.00 2.0000)
          -----> GR (12.00 2.0000)
          -----> ADRI (10.00 2.0000)
          <----- GR (12.00 2.0000)
          -----> CBF (2) (16.00 1.6000)
          -----> SRF (16.00 1.6000)
          <----- Pit-la (14.00 2.0000)

00101  GGAGTGGGAGCAGAGAGTTCCTGAAGCTCACTGGCTAGTATTCTTGCTA
          <---- GR (10.00 2.0000)
          -----> AGP/EBP (10.00 1.1111)
          -----> C/EBP (11.00 1.2222)
          -----> C/EBP (11.00 1.2222)
          -----> C/EBP (11.00 1.2222)
          -----> C/EBP (11.00 1.2222)
          -----> C/EBP (11.00 1.2222)
          -----> LAP (10.00 1.1111)
          -----> NF-IL6 (10.00 1.1111)
          -----> NF-IL6beta (10.00 1.1111)
          <----- CP2 (10.00 0.9091)
          -----> CPIA (14.00 2.0000)

```

FIG. 13D

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-----> c-Ets-1 54 (11.00 1.8333)
-----> c-Ets-1 68 (11.00 1.8333)
-----> c-Ets-2 58-64 (11.00 1.8333)
-----> LAP (10.00 1.1111)
-----> NF-IL6 (10.00 1.1111)
-----> NF-IL6beta (10.00 1.1111)
-----> PEA3 (11.00 1.8333)
-----> PEA3 (11.00 1.8333)
-----> PEA3 (11.00 1.8333)
<----- kappaY factor (16.00 2.0000)
<----- c-Ets-2 (12.00 2.0000)
-----> alpha-CBF (10.00 2.0000)
-----> alpha-CP1 (10.00 2.0000)
-----> alpha-CP2A, alpha-CP2b (10.00 2.0000)
-----> alpha-IRP (10.00 2.0000)
-----> CDP (10.00 2.0000)
-----> CDP2 (10.00 2.0000)
-----> Clox (10.00 2.0000)
-----> CP1 (10.00 2.0000)
-----> CP1 (10.00 2.0000)
-----> CP2 (10.00 2.0000)
-----> Cux (10.00 2.0000)
-----> H1TF2 (10.00 2.0000)
-----> NF-1 (10.00 2.0000)
-----> NF-E (10.00 2.0000)
-----> NF-E (10.00 2.0000)
-----> SRF (10.00 2.0000)
-----> TGGCA-binding protein (10.00 2.0000)
<----- C/EBP (10.00 2.0000)
<----- CBF (1) (10.00 2.0000)
<----- CBF (2) (10.00 2.0000)
<----- CBF-A (10.00 2.0000)
<----- CBF-B (10.00 2.0000)
<----- CCAAT-binding factor (10.00 2.0000)
<----- CDF (10.00 2.0000)
<----- CRF (10.00 2.0000)
<----- CTF (10.00 2.0000)
<----- NF-Y (10.00 2.0000)
<----- NF-Y' (10.00 2.0000)
-----> HOXA5 (10.00 1.0000)
-----> Hb (16.00 1.6000)
-----> GATA-1 (12.00 2.0000)
<----- GATA-1 (10.00 1.6667)
<----- GATA-1 (10.00 1.6667)
<----- GATA-1 (10.00 1.6667)
<----- GATA-2 (10.00 1.6667)
<----- GATA-2 (10.00 1.6667)
<----- GATA-2 (10.00 1.6667)

```

FIG. 13E

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```

<----- GATA-2 (12.00 2.0000)
<----- GATA-3 (10.00 1.6667)
<----- GATA-3 (10.00 1.6667)
<----- GATA-3 (10.00 1.6667)
<----- GATA-3 (10.00 1.6667)
<----- GATA-3 (10.00 1.6667)
<----- NF-E1b (10.00 1.6667)
<----- NF-E1c (10.00 1.6667)
----> DBF-A (10.00 2.0000)
<---- GAL4 (10.00 2.0000)
<---- TBP (10.00 2.0000)
00451 ACATTATGAGCAGCCTGTTGCCAGCACATAAGGGATGTGGAGTATGAGAA
      <----> NF-1/L (10.00 2.0000)
      <----> GCR1 (10.00 2.0000)
      <-----> C/EBP (14.00 1.7500)
      <-----> C/EBP (14.00 1.7500)
      <-----> C/EBP (14.00 1.7500)
      <-----> C/EBP (14.00 1.7500)
      <-----> C/EBP (14.00 1.7500)
00501 GCGTGAAAAGGGTAAATCAAAGATAATTAATTTGATGGTAATTCAC
      <----- AGP/EBP (13.00 1.8571)
      <-----> H1NF-A (12.00 1.0000)
      <-----> Kr (16.00 1.6000)
      <----> Pit-la (10.00 2.0000)
      <----> GATA-1 (12.00 2.0000)
      <----- GATA-1 (10.00 1.6667)
      <----- GATA-1 (10.00 1.6667)
      <----- GATA-1 (10.00 1.6667)
      <----- GATA-2 (10.00 1.6667)
      <----- GATA-2 (10.00 1.6667)
      <----- GATA-2 (10.00 1.6667)
      <----- GATA-2 (12.00 2.0000)
      <----- GATA-3 (10.00 1.6667)
      <----- GATA-3 (10.00 1.6667)
      <----- GATA-3 (10.00 1.6667)
      <----- GATA-3 (10.00 1.6667)
      <----- GATA-3 (10.00 1.6667)
      <----- NF-E1b (10.00 1.6667)
      <----- NF-E1c (10.00 1.6667)
      <-----> DBF-A (10.00 2.0000)
      <-----> HOXA5 (10.00 1.0000)
      <-----> GAL4 (10.00 2.0000)
      <-----> TBP (10.00 2.0000)
      <-----> N-Oct-3 (12.00 1.7143)
      <-----> N-Oct-3 (12.00 1.7143)
      <-----> N-Oct-3 (12.00 1.7143)
00551 AGTTTGTAGTTAGCTGCCTGTGCTTTAGCCAGAAAATGCGTAGGCCTGC
      <----> Zeste (10.00 2.0000)

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FIG. 13F

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----> Zeste (10.00 2.0000)
      <----- CP2 (11.00 1.0000)
            -----> Ker1 (16.00 1.6000)
            <---- Lvc (10.00 2.0000)
00601 AGGTATCCAAGAAGTACAATTCCAGAAAGTCCGCAGTGCAGGCTCTGGGC
      -----> GR (12.00 2.0000)
            -----> AGP/EBP (13.00 1.8571)
            -----> IL-6 RE-BP (18.00 2.0000)
            -----> IL-6 RE-BP (18.00 2.0000)
            <----- Spl (16.00 1.6000)
            -----> Spl (10.00 2.0000)
            -----> Lvc (10.00 2.0000)
            -----> GAL4 (10.00 2.0000)
            -----> GR (10.00 2.0000)
            <---- T-Ag (10.00 2.0000)
            -----> GCF (10.00 1.4286)
            <----- Elk-1 (10.00 1.0000)
            <----- c-Ets-1 (16.00 1.6000)
            <----- c-Ets-1 54 (12.00 1.5000)
            <----- c-Ets-1 68 (12.00 1.5000)
            <----- c-Ets-1 (12.00 1.5000)
            <----- PEA3 (12.00 1.5000)
            <----- PEA3 (12.00 1.5000)
            <----- PEA3 (12.00 1.5000)
            -----> Bcd (16.00 1.6000)
            <----- E1A-F (14.00 2.0000)
00651 CGGATGTAGTCTTGGTCTGAGAGCTGCTGGTCCAAGCTGGGCAAGGTCTC
      -----> GCR1 (10.00 2.0000)
            <----- EF1I (18.00 1.6364)
            <---- H4TF-2 (10.00 2.0000)
            <----- GCF (10.00 1.0000)
            <---- T-Ag (10.00 2.0000)
            <---- LF-A1 (10.00 2.0000)
            <----- ELP (14.00 2.0000)
            <---- ADR1 (10.00 2.0000)
00701 CCACGTCTACATTC
  
```